TECH NOTE

Mastering the Mix: Your Sunny Selection Guide

We don't want to lock you down into one Sunny device. You want gentle laminar flow? Sure! Chaotic? It's your call. From Micromix to cross mix, flow focus and beyond, our lineup of Sunnies - ultra-smooth, high-quality, high-performance, reusable microfluidic mixers – are here to back you up. Not sure what mixing type fits your formulations? No worries! We've crafted the ultimate guide to the mixing world. Throw on your Sunnies and follow us in!

Mixing Technologies

LNPs are formed when lipids in an organic solvent and nucleic acids in an aqueous buffer are mixed. But what's the scoop on mixing? It's pretty simple: faster mixing means smaller particles, and uniform mixing means a tighter distribution.

Particles self-assemble as the environment changes around them (Figure 1). Firstly, there is an electrostatic interaction of the positively charged ionizable lipids and the negatively charged nucleic acids. Then the rest of the lipids assemble around these nuclei, driven by hydrophobic interactions, as the lipids' environment becomes more polarized due to its mixing with water.

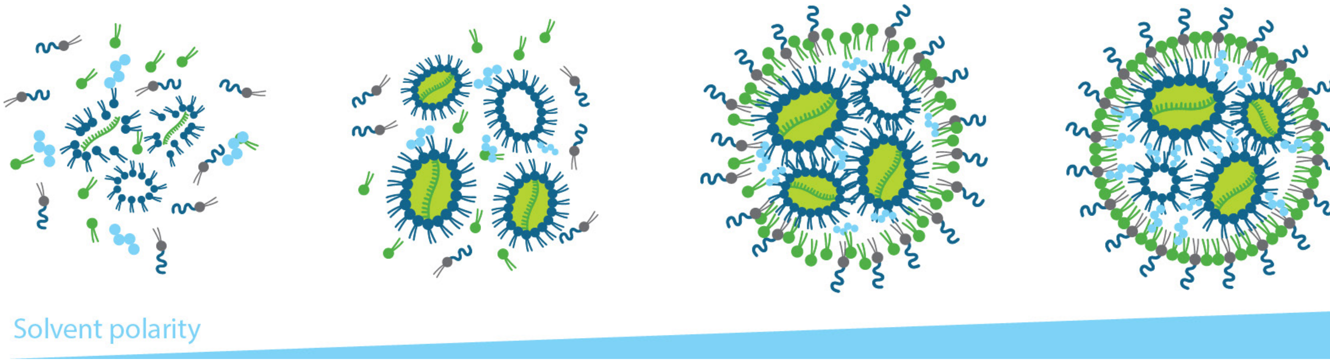

Figure 1: Illustration showing the self-assembly of an LNP during the mixing process.

The longer this process takes, the more time these particles have to grow. You'll see this with most formulations, including LNPs and liposomes (Figure 2). As you increase the total flow rate, the particle size will drop as the time it takes for the materials to mix decreases (Figure 3).

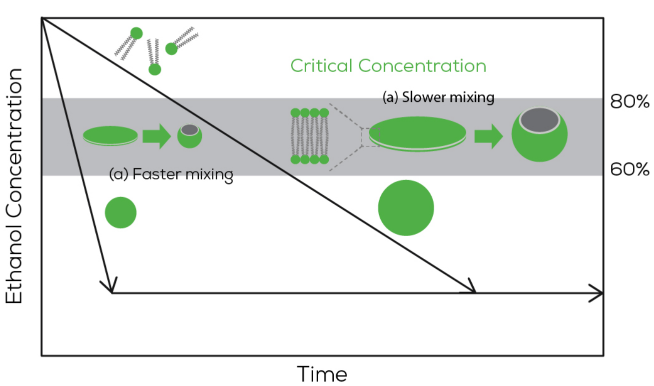

Figure 2: Effect of mixing speed on particle formation.

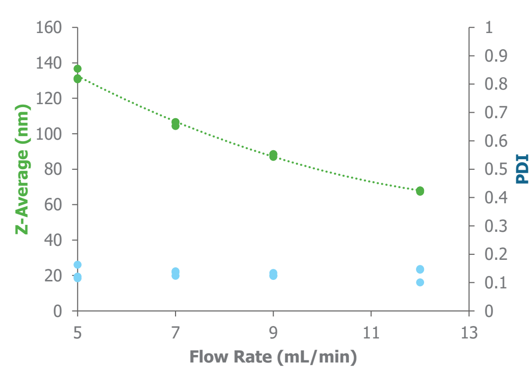

Figure 3: Effect of total flow rate (TFR) on LNP size.

When this process is uneven, particle size distribution will increase, as the mixing speed will vary at different points. This causes variations in particle size. That's where microfluidics step in – unrivalled mastery of flows and mixing channels, guaranteeing a flawless and precise mix every single time.

So, what are the different types of mixing, and what are they best for?

Laminar and chaotic mixing

In laminar mixing, materials flow in a smooth, predictable manner, with layers of material easily slipping past one another. The mixing of two streams is driven by the diffusion of material into the neighbouring stream, and so is proportionate to the diffusion constants and contact area of the materials. We use hydrodynamic flow focusing (HFF), a version of laminar mixing which uses two streams of fluid flow to compress a central flow (Figure 4). The compression of this flow means the distance necessary for diffusion is reduced and so mixing is accelerated. Particle size is related to the compressed stream width, which is affected by channel size and flow rates.

Figure 4: Comparison of stable laminar flow mixing (left) and chaotic mixing (right).

Due to the laminar flow, this method is low pressure and highly controllable, giving access to fine control of particle properties and excellent PDIs.

Because turbulent flow is induced by high flow rates and wide channel sizes, genuine turbulent flow is rarely reached in microfluidics, and the lateral mixing regimes are created by mixing and geometries, such as flow spitting and channel expansion. This is called 'induced chaotic flow'.

Induced chaotic flow is dominated by eddies or vortices as fluids travel down the channel, leading to significant lateral mixing. Typically, fine control is sacrificed for the most rapid mixing speeds as the eddies that create lateral mixing across the channel mean mixing timescales are very short, resulting in smaller particles.

The Sunnies

Sunny 490 Trident T

The Sunny 490 Trident T (Figure 6) is the ultimate in HFF having wide channels for comparatively low flow velocities and back-pressures. With a built-in dilution channel for ease of use, the Trident T is an excellent choice for those long, high-throughput screening runs. Due to the highly controlled laminar mixing, the control over particle size is high over the whole flow rate range of the Sunny, with the smallest sizes being achieved at the highest flow rates (Figure 5).

The steady drop in particle size across the flow rates tested is a good indicator of continued laminar flow.

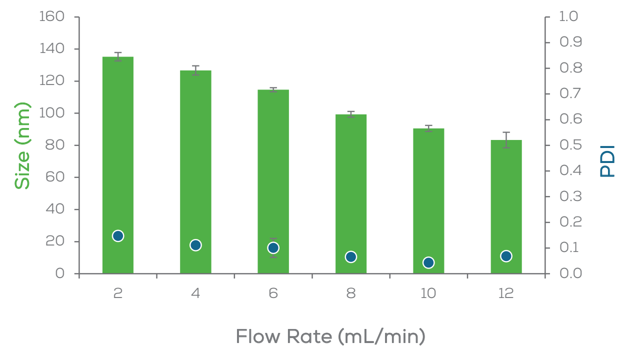

Figure 5: Sunny 490 Trident T flow rate screen.

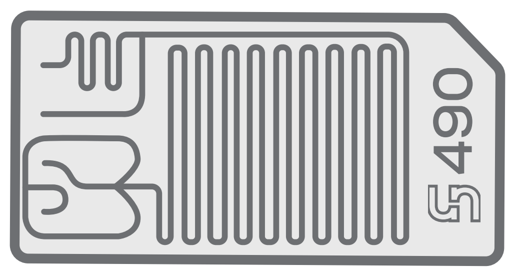

Figure 6: Sunny 490 Trident T.

Sunny 150 3D

The Sunny 150 3D (Figure 8) is an advanced microfluidic mixer design that utilizes 3D flow focusing, by passing the central input though a pore, rather than a full-size channel. This allows the flow to be compressed in all directions, not just from either size, bringing exquisite control to the mixing process (Figure 7).

This Sunny also comes with the option of a simultaneous, outer dilution sheath flow, further compressing both original flows for faster mixing.

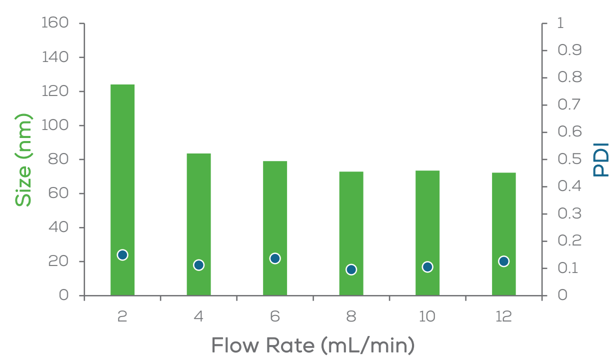

Figure 7: Sunny 150 3D flow rate screen.

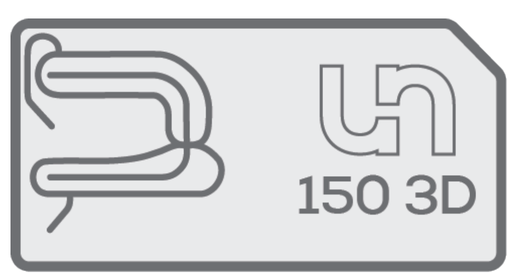

Figure 8: Sunny 150 3D.

Sunny 275X, 190X and 100X

The Sunny X splits the aqueous flow on the chip and recombines it with the organic flow at a narrow junction which compresses the flow. The channel then expands, causing chaotic flow and accelerating the mixing process. The particle sizes are lower as a result of this and drop quickly as the flow regime moves from laminar at lower flow rates to chaotic. This Sunny is available in three junction sizes – 100 µm, 190 µm and 275 µm.

These Sunnies produce small particles, reaching minimum sizes at total flow rates between 6-10 mL/min. The Sunny 100 X (Figure 10) reaches this size at a lower flow rate due to the smaller junction size, while the Sunny 190 X has a shallower curve as it takes a higher flow rate to reach the fully chaotic flow state. The 275 X produces the largest particles, but it can reach the highest flow rates (Figure 9).

These Sunnies are ideal both for screening and for longer runs, and the different channel sizes mean it's easy to optimize a process as you scale up.

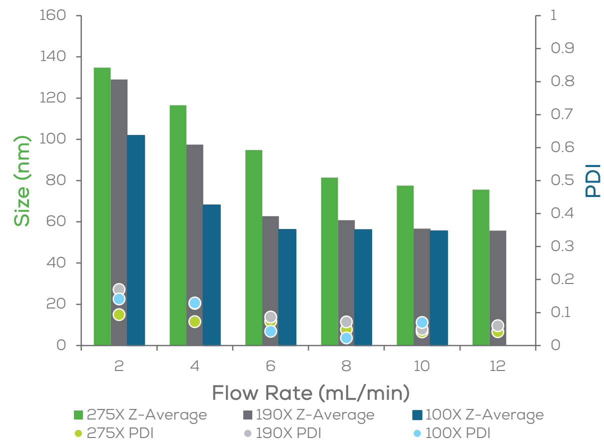

Figure 9: Sunny 275X, Sunny 190X, and Sunny 100X flow rate screens.

Figure 10: Sunny 100X.

Sunny 275T, 190T and 100T

The Sunny T can be used as your diluent input channel, or as a mixing chip in its own right. The Sunny T is has probably the most straight forward design of all the chips with the two flows meeting at a 90 degree angle (or head-on, if desired). It still has the compression at the junction, to ensure excellent and thorough mixing (Figure 11).

Figure 11: Sunny 100T.

The trend in particle size across the Sunny T range follows that of the Sunny X range and is also available in three junction sizes – 100 µm, 190 µm and 275 µm (Figure 12).

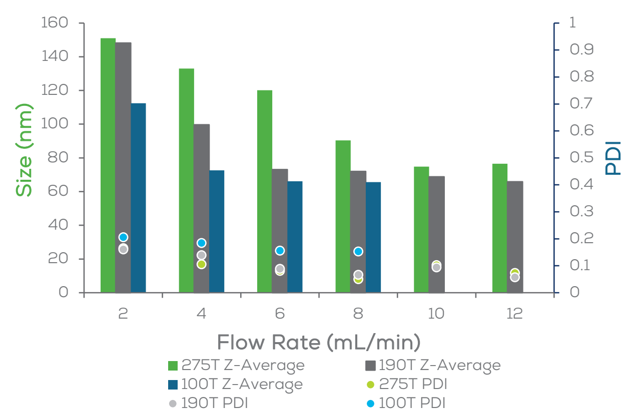

Figure 12: Sunny 275T, Sunny 190T, and Sunny 100T flow rate screens.

Sunny 50 Micromixer

The Sunny 50 Micromixer (Figure 14). is designed to produce highly chaotic flow at all flow rates – fast or slow! It uses a flow-focussing junction, after which the flow is split into multiple channels; recombining and splitting repeatedly along the length of the mixing channel.

This results in low particle sizes even at low flow rates, suggesting that the mixing geometry induces chaotic flow regardless of flow velocity, unlike the Trident T or XT Sunnies. The complex geometry gives it a high back-pressure with a more limited flow rate range (Figure 13).

We would recommend this for formulation screening or for targeting the smallest possible size for a challenging particle formulation.

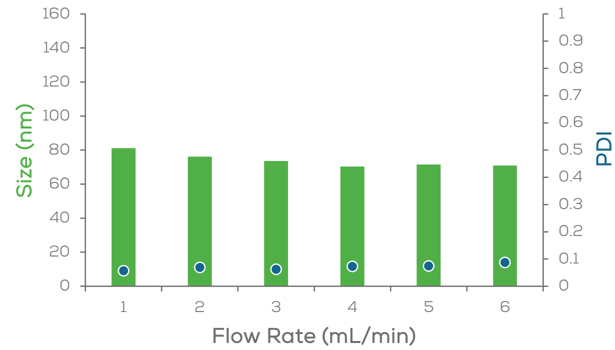

Figure 13: Sunny 50 Micromixer flow rate screen.

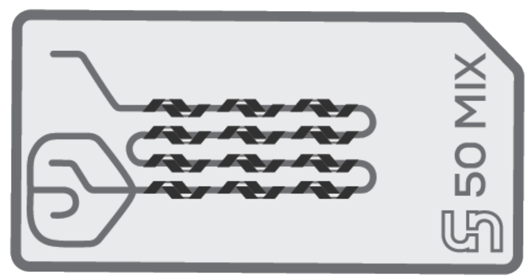

Figure 14: Sunny 50 Micromixer.

Summary

Your formulation is unique, and finding the perfect match depends on factors like reagent concentration, nucleic acids construct, lipid composition and T_m, and your specific workflow nuances – particle size, flow rate ratios and total flow rates, to name a few. It's time to dive in and discover the Sunny that rocks your mix (Figure 15).

*All data based on SM-102 Lipid Exploration Kit from Cayman Chemicals: Lipids dissolved in ethanol at a total concentration of 10 mM, with a molar ratio for SM-102/DSPC/Cholesterol/DMG-PEG2000 of 50/10/38.5/1.5. NP ratio was 6:1, and the cargo was polyA (>200nt). Flow rate ratios were 3:1 aqueous:organic in all cases.

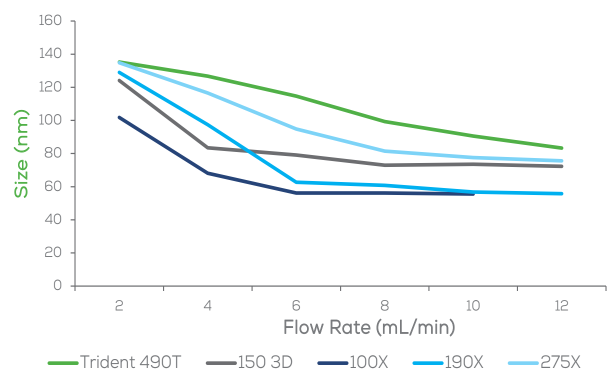

Figure 15: Flow rate screens for the Sunny range.

UNCHAINED LABS

Unchained Labs
4747 Willow Rd
Pleasanton, CA 94588
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com